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(54) Title: METHOD OF FAVORABLY MODIFYING POULTRY INTESTINAL MICROFLORA

(57) Abstract

Bacillus pumilus, *Bacillus coagulans*, or mixtures thereof in spore form are fed to poultry to modify their intestinal microflora. Potentially harmful coliform organisms are reduced, and other advantageous results are obtained, including improved poultry health, reduced mortality, improved litter quality, and improved feed utilization.

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METHOD OF FAVORABLY MODIFYING POULTRY INTESTINAL MICROFLORA

FIELD OF INVENTION

The general field of this invention is direct-fed microbials for animal feeding. The present invention is more particularly concerned with direct-fed microbials for poultry.

BACKGROUND OF INVENTION

Direct-fed microbials (commonly known as probiotics) are live microbial cultures supplemented into feeds which contribute well-being to animals by improving their intestinal microbial balance. All direct-fed microbials have as their active ingredient live bacterial cells. Species of the genera Lactobacillus, Streptococcus, and Bifidobacterium have been employed as direct-fed microbials. The major mode of action for these bacteria is production of organic acids especially lactic acid which can inhibit growth of pathogenic or potentially pathogenic bacteria. Other benefits from direct-fed microbials are a reduction of the oxidation/reduction potential in the gut, deconjugation of the bile acids, production of antimicrobial substances like hydrogen peroxide, and secretion of enzymes which may assist the digestive process.

Modifying the intestinal microflora of poultry, such as chickens, turkeys and ducks, presents special problems that are different than those encountered with domestic mammals, such as cattle, sheep and swine. Coliform organisms are ubiquitous in the intestines of poultry, and if their growth is not controlled, the coliforms can multiply and have adverse effects on the health and growth of poultry.

Poultry are also especially subject to infection with Coccidia which can produce intestinal lesions which predisposes the bird to coliform proliferation. For this reason, coccidiostats are commonly included in poultry feeds. It is therefore important for a poultry bacterial feed additive to be compatible with coccidiostats.

Bacterial additives in poultry feeds have not been extensively studied. However, it has been reported that some benefit can be obtained by including Bacillus subtilis cells in turkey feeds: Jaraphocakul, et al. (1990), Poultry Sci., 69:1966-1973. In one experiment with B. subtilis it was found that body weight and feed efficiency of the turkeys were not significantly affected. Microbiological testing disclosed that the B. subtilis counts in the crop and cecum were increased by the feeding, but the B. subtilis feeding failed to influence intestinal Lactobacillus or Escherichia coli. In the second experiment, limited increases in body weight and feed efficiency were observed. Turkeys receiving the B. subtilis showed an increase in body weight gain at 12 weeks, and the feed efficiency showed improvement at 20 weeks.

Chr. Hansen's Laboratory, Inc. of Milwaukee, Wisconsin, USA has marketed a direct-fed microbial additive under the trademark "BIOMATE 2B", which contains a mixture of Bacillus subtilis and Bacillus licheniformis. That additive has been used with domestic animals including poultry. Chr. Hansen's Laboratory has carried out research investigations to find a lactic acid-producing bacteria which is especially beneficial when fed in a mash or pelletized

feed to poultry. The present invention is a result of that research investigation.

U.S. Patent 4,919,936 of 1990 discloses a strain of Bacillus subtilis (C-3102; FERM BP-1096), which is said to provide improved results with a wide variety of domestic animals including poultry. Example 3 (cols. 4-5) of this patent reports experiments with broilers and laying chickens. An increase in body weight and feed conversion was obtained for the broilers, and the number of eggs and egg weights were increased for the laying chickens. No examinations of intestinal microflora were reported.

U.S. Patent 4,999,193 of 1991 discloses a strain of Bacillus cerus (IP5832) for improving the growth of domestic animals. According to this patent, B. cerus forms vegetative spores which can be incorporated in pelletized feeds, the spores being sufficiently heat resistent to survive a pelletizing process. The claimed beneficial effects are improvements in feed conversion and growth. Example 3 (cols. 3-4) of this patent describes an experiment with female chicks where the live weight and consumption index was improved by the feeding of the B. cerus strain. No data relating to intestinal microflora was presented.

Bacillus pumilus is a spore-forming lactic acid-producing bacteria, which produces acetylmethyl-carbinol, coagulates milk, and ferments gelatin, and does not grow well at 60°C. Bacillus coagulans is also a spore-forming, lactic acid producing bacteria, which also produces acetylmethylcarbinol, coagulates milk, grows variably at 60°C, but does not ferment

gelatin. The properties of B. pumilus or B. coagulans as direct-fed microbial feed additives have not been previously reported. As far as it is known neither B. pumilus nor B. coagulans have been used in a direct-fed microbial feed prior to the research studies of Chr. Hansen's Laboratory.

SUMMARY OF INVENTION

In research studies which led to the present invention, it was found that Bacillus pumilus has properties which make it especially desirable for incorporation in a direct-fed microbial poultry feed. B. pumilus has the ability to produce lactic acid after being pelleted in a feed and has other important properties. B. pumilus can withstand acid conditions, and can germinate quickly from spores in the intestines of poultry. This contrasts with bacteria from the genera Lactobacillus, Streptococcus, and Bifidobacteria which do not appreciably produce lactic acid after being pelleted in a feed.

By incorporating B. pumilus spores in poultry feeds, the intestinal microflora can be favorably regulated. Among other observed results, intestinal coliform organisms can be appreciably reduced. It has also been found that B. pumilus and/or B. coagulans can be incorporated in poultry feeds containing coccidiostats for conjoint action. Not only does B. pumilus and coccidiostats not interfere with each other's actions, but they appear to somewhat act synergistically in preventing formation of lesions due to coccidia, and in improving the health of the birds, resulting in reduction in mortality. Feed efficiency and growth rate are also significantly improved. Further, due to the enzymatic action of B. pumilus on

the ingested feed, litter quality is improved as evidenced by less wet, caked litter, better litter score, and lighter litter weight.

Summarizing, the method of the present invention in which B. pumilus spores are incorporated in poultry feeds can improve health, reduce mortality, improve litter quality, and at the same time improved feed utilization. A further advantage is that these spores can be conveniently administered. For example, the spores can be premixed with a phosphate salt which is to be incorporated in the feed, the phosphate salt acting as a diluent and carrier for the spores. It is not necessary to contol the water activity of the phosphate salt because the bacteria are in spore form. The spores, with or without premixing with a carrier, can be incorporated in pelletized feeds.

DETAILED DESCRIPTION

Bacillus pumilus is the preferred bacteria for use in practicing the method of this invention. Alternatively or additionally, Bacillus coagulans can be used. Both species are spore-forming and produce lactic acid. The direct-fed microbial properties of B. pumilus has not been considered prior to this invention, although it is a Bacillus species that can be ingested by domestic animals. Usable strains of B. pumilus or B. coagulans are available from public depositories, such as the Northern Regional Research Laboratory, U.S.D.A., Peoria, Illinois, USA. Available strains from this source include Bacillus pumilus NRRL NRS-272 and Bacillus coagulans NRRL-B768. These strains are illustrative but are not limiting. For practicing the present invention, other lactic acid-producing spore-forming strains of

Bacillus pumilus or B. coagulans can be obtained and used. A presently preferred strain if Bacillus pumilus was designated "BC 235" by Chr. Hansen's Laboratories, Inc., Milwaukee, Wisconsin. This strain has been placed on deposit with the Northern Regional Research Laboratory pursuant to the provision of the Budapest Treaty. This strain has been assigned the number NRRL B-21013. The BC 235 strain has been found to have the characteristics of the species of B. pumilus; viz. it does not grow at 60°C (growth stopping at 55°C), it does not grow well anaerobically, and it ferments gelatin, and has other characteristics by typing.

In practicing the method of this invention, the poultry feed is modified by incorporating an effective concentration of viable spores of Bacillus pumilus and/or coagulans. The modified feed is then fed to poultry in an amount and for a length of time sufficient to appreciably modify their intestinal microflora. This modification of intestinal microflora can be verified by microbial examination of the intestinal contents of killed birds, for example, by determining that a reduction in coliform organisms has occurred. For practical commercial feeding purposes the amounts to be fed and the feeding periods can predetermined and standardized.

The modified feeds used for the purpose of the present invention can contain from 10^3 to 10^8 viable spores per gram of feed. In presently preferred embodiments, the feeds contain from 10^5 to 10^7 viable spores per gram of feed. For example, a preferred level for commercial use is approximately one million spores per gram of feed.

The method can be practiced with a wide variety of poultry being raised for commercial purposes. Important species include chickens, turkeys, and ducks. The method can be used with particular advantage with poultry being raised for meat production, but its beneficial effects are not limited to this class of birds. The method is generally applicable to male, female and caponized birds, and can be used effectively with breeder birds and egg laying birds. Further, the benefits of the method are not limited to a particular growth phase, but can be used advantageously with starter, grower and finisher birds.

To maximize the benefits of the method of the present invention, it is preferred to administer the modified feeds on a daily basis, for example, continuously for several weeks. Preferably, feeding is continued for at least three weeks, such as from one day of age to three weeks of age. However, the length of feeding will depend on the kind of poultry and the purpose for which they are being fed.

Propagation of Bacillus pumilus or coagulans can be carried out according to known methods for growing Bacillus species. For example, stock cultures may be propagated in a trypticase soy broth fortified with yeast extract and minerals. The propagation medium can be adjusted initially to a neutral pH. Fermentation is carried out with aeration and agitation, at a temperature of around 37°C, and pH control is preferably used. In the first part of the fermentation, the cells go through a logarithmic growth phase. After the nutrients begin to become depleted, the cells convert to spore form. Then the

spores can be harvested by centrifugation and lyophilized for incorporation in poultry feeds.

The spores can be admixed with a suitable carrier for addition to the feed ingredients. For example, a phosphate salt can be used as a carrier, since phosphate salts are necessary ingredients of poultry feeds. The phosphate salts do not need to be prepared in any special way. For example, spores can be mixed with commercial phosphates such as dicalcium phosphate, defluorinated phosphate, etc. The desirable proportions of spores to phosphate salts range from about 10^6 to 10^8 spores per gram of phosphate salt.

Poultry feeds are usually designed as complete rations. These feeds comprise mixtures of solid feed ingredients in the form of powders or granules. When the feed mixture is to be pelletized, a liquid ingredient may be included such as molasses. The spores should be blended substantially uniformly with the feed ingredients. Thereafter, if desired, the feed can be pelletized, or it can be fed as a loose or mash-type feed.

The practice of this invention and the results which can be obtained thereby are further illustrated by the following examples:

Example I

Propagation of Bacillus pumilus or coagulans can be carried out to obtain the bacteria in spore form. For example, stock cultures are propagated overnight at 37°C in trypticase soy broth before being inoculated into production medium. Production medium can consist of meat peptone (2%), yeast extract (0.5%), K_2HPO_4 (0.1%), $MgCl_2 \cdot 6 H_2O$)

(0.02%), $MnCl_2 \cdot 4 H_2O$ (0.01%), $CaCl_2$ (0.02%), and defoamer (0.1%); pH was adjusted to 7.0 using 50% NaOH. Parameters of the fermentation are 37°C, 0.5 vvm aeration, agitation to maintain greater than 25% dissolved oxygen, and pH was controlled with 25% H_2SO_4 or 50% NaOH. A dextrose (50%) feed is used during fermentation with a delivery rate of 3.8 gram per hour for the first 16 hours of fermentation. The fermentation is terminated when the culture is considered to be completely sporulated as determined by vesical microscopic examination. The spores thus produced are separated from the fermentate by high speed centrifugation and lyophilized.

Example II

Purpose: To investigate the effect of Hansen's BC 235 viable spores on improving growth performance, decreasing mortality and modifying intestinal microflora in turkey poults.

Test Methods: Day old turkey poults were weighed, wing banded and then placed in their appropriate pens. Treatments included: 1) a typical starter turkey ration (control diet) and 2) this same diet but containing viable BC-235 as a feed additive. The spores were added to the control diet by using dicalcium phosphorus as a carrier, the spores being premixed with the carrier. Approximately 1 trillion spores were added per ton (2000 lbs.) of feed. Birds were observed daily for signs of morbidity and(or) mortality and weighed at 7, 14, and 21 days of age. At the conclusion of the trial, representative intestinal samples were enumerated for intestinal bacteria.

Test Results: Mortality was reduced in birds fed the BC 235 spores when compared to the control birds (0.0% vs. 4.2%). Total body weight gain for the 21 day test period was greater for the birds fed the feed additive (23.32 lb. vs. 22.79). Feed efficiency for the 21 day period was improved in birds fed the feed additive (1.781 vs. 1.538). The spores decreased the concentration of intestinal coliforms from 12 million coliforms per gram of wet intestinal contents to 3 million coliforms per gram of wet intestinal contents. The intestinal spore count, an indicator of the presence of BC 235 spores, was increased in birds fed the feed additive from 7100 spores per gram of wet intestinal contents in the control birds to 190,000 spores per gram of wet intestinal content in the treated birds.

Summary: These findings indicate that feeding the BC 235 spores to turkey poults increased weight gain, improved feed efficiency, decreased mortality, decreased the intestinal coliform population, providing less enteric pathogen stress, and that the BC 235 spores were viable in the intestinal tract.

Example III

Purpose: To investigate the effect of feeding Hansen's BC 235 spores to chickens. The investigation included a study of growth performance, litter quality, pasted vents, survivability of the spores in the intestinal tract of broiler chickens, and the viability of the spores in the diet after pelleting at 180°F.

Test Methods: Day old chicks were assigned to one of 48 pens and to one of 8 treatments. Dietary treatments were a control diet to which one of four different coccidiostats were added (amprolium, monensin, salinomycin, or maduramicin). The other four dietary treatments were these aforementioned diets with the BC 235 spores as an additive. The spores were added to the diet via a non-conditioned, defluorinated phosphate carrier, being premixed therewith, to deliver approximately 1 trillion spores per ton (2000 lbs.) of feed. Birds were weighed at 21, 42 and 47 days of age. At day 10 of the study, birds were evaluated for the incidence of pasted vents. At day 14, all birds were orally challenged with coccidia and then on day 21, representative birds were evaluated for intestinal lesions. On days 14, 21 and 47, representative birds were evaluated for intestinal spore counts (detection of the feed additive) and coliforms. At the conclusion of trial, litter quality was evaluated.

Test Results: Data are pooled across coccidiostats to compare the overall effect of feeding the feed additive to broilers. At day 42 of the trial, the BC 235 additive improved feed conversion compared to the diets not containing the feed additive (1.829 vs. 1.841) and tended to improve the overall feed conversion at day 47 (1.911 vs. 1.920). The feed additive decreased mortality from 3.05% to 2.01% and reduced the incidence of intestinal lesions caused by the coccidia challenge from 1.96 to 1.665 (an average score across all intestinal sections). The feed additive also improved litter quality as evidenced by less percentage of wet, caked litter (47.9% vs.

48.7%), better litter score (1.6 vs. 1.8), and a lighter litter weight (57.4 kg. vs. 59.1 kg). Further, the feed additive reduced the incidence of pasted vents (sticky droppings) in 10 day old chicks from 1.76 to 1.52. The intestinal spore data on day 47 indicated that the BC-235 spores were viable in the intestinal tract (11,000 spores per gram of wet intestinal conents vs. 6,000 spores per gram of wet intestinal contents), and the coliform population reduced by 25% (9.5 million per gram of wet intestinal contents vs. 12.5 million per gram of wet intestinal contents). The pelleting data indicated that BC 235 spores survived pelleting at 180°F.

Summary: The findings of this study indicate that feeding BC 235 spores to broiler chickens improved feed efficiency, reduced mortality, improved litter quality, decreased the incidence of pasted vents. Moreover, the
BC 235 spores were viable in the intestinal tract in the presence of coccidiostats, reduced intestinal coliform counts, when administered in feeds pelleted at 180°F.

Example IV

B. pumilus or coagulans spores can be fed to poultry in conjunction with other helpful bacteria. For example, Bacillus licheniformis and Bacillus subtilis strains can be used to produce significant amounts of amylase and protease enzymes in the poultry intestines. For example, a combined direct-fed microbial additive can be prepared as follows:

Composition of the feed additive per 1000 g:	
Bacillus subtilis spores (100×10^9 bacteria/g)	50 g
Bacillus licheniformis spores (100×10^9 bacteria/g)	50 g
Bacillus pumilus or coagulans spores (100×10^9 bacteria/g)	100 g
Sweet whey powdeR	800 g

The feed additive of the foregoing composition was used in a comparative study of chicks of older hens and chicks of younger hens.

Test Methods: One thousand and eighty feather-sexed broiler chicks were used in this study. Half of the chicks were from hens 33 weeks old and half were from hens which were sixty weeks of age. Chicks were distributed evenly throughout the pens. Chicks from old hens were separated from chicks with young hens. Brooding heat was provided by infrared heat lamps in each pen, with standard brooding temperatures being maintained throughout the trial. Pen floors were covered with 7 cm of used wood shaving topdressed with 2 to 3 cm of new wood shavings. Pens were provided with an automatic bell-type waterer and a tube-type feeder. Chicks were vaccinated for Marek's disease and debeaked at the hatchery, then given no further vaccinations, as per standard practice for that area.

Feeds were formulated and mixed in batches sufficient for one week's worth of feeding. Diets were formulated to meet National Research Council recommendations. All starter and grower feeds contained Amprol Plus at a rate of 0.0125% amprolium

and 0.0004% ethopabate. Withdrawal feed, which was f d for the final three days of the trial, contained no medication. The feed additive as described above with BC 235 spores was mixed with the feed prior to pelleting (180°F) at a rate of 2×10^{11} viable bacteria per ton of feed. Broiler starter feed was fed from day 0 to 21, grower/finisher feed was fed from day 21 to day 39, and withdrawal feed from day 39 to 42.

Test Results: Chicks from old hens responded to the feed additive with lower mortality rates than control chicks. Treated chicks from younger hens did not show lower mortalities until week 6. Relatively little effect was observed on final body weights, feed intake, or feed efficiency. This test showed a benefit of the present invention in lowering mortality in broiler chicks.

CLAIMS

We claim:

1. The method of favorably modifying intestinal microflora of poultry, comprising incorporating in a poultry feed an effective concentration of viable spores of Bacillus pumilus, Bacillus coagulans, or admixtures thereof and feeding the spore-containing feed to poultry in an amount and for a length of time sufficient to appreciably modify their intestinal microflora.

2. The method of claim 1 in which said sprores are Bacillus pumilus.

3. The method of claims 1 or 2 in which said feed contains from 10^3 to 10^8 viable spores per gram of feed.

4. The method of claims 1 or 2 in which said poultry are fed said modified feed for at least three weeks.

5. The method of claims 1 or 2 in which said modified feed contains from 10^5 to 10^7 viable spores per gram of feed, and said poultry are fed said modified feed on a daily basis for at least three weeks.

6. The method of claims 1, 2, 3, 4 or 5 in which said poultry are chickens.

7. The method of claims 1, 2, 3, 4 or 5 in which said poultry are turkeys.

8. The method of claims 1, 2, 3, 4 or 5 in which said poultry are chickens, and said modified feed is a mixture of feed solids which are pelleted before being fed.

9. The method of claims 1, 2, 3, 4 or 5 in which said poultry are turkeys, and said modified feed is a mixture of feed solids which are pelleted before being fed.

10. The method of favorably modifying intestinal microflora of poultry, comprising incorporating in a mixture of poultry feed solids from 10^5 to 10^7 viable spores of Bacillus pumilus per gram of feed, and feeding the modified feed to chickens or turkeys daily for at least three weeks.

11. The method of claim 1 in which said spores are mixed with a carrier comprising a phosphate feed additive in powder form, and thereafter the spore-containing phosphate feed additive is employed to modify the poultry feed by incorporation therein.

12. The method of claim 10 in which said spores are mixed with a carrier comprising a phosphate feed additive in powder form, and thereafter the spore-containing phosphate feed additive is employed to modify the poultry feed by incorporation therein.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 1/20; A23K 1/00; A01N 63/00
US CL :424/93K; 435/252.5; 426/61

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93K; 435/252.5, 832; 426/61

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Chemical Abstracts, CABA, CJACS, INPADOC, EMBASE, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,999,193 (Nguyen) 12 March 1991, See Entire Document	1-11
Y	FR, A, 1,502,961 (Sankyo Co. Limited) 24 November 1967, See Entire Document	1-11
Y	CS, A, 232,777 (Dedek, et al.) 17 July 1984, See Entire Document	1-11
A	US, A, 3,903,263 (Mann) 02 September 1975	1-11
A	US, A, 3,124,517 (Eloy) 10 March 1964	1-11
A	US, A, 4,919,936 (Iwanami, et al.) 24 April 1990	1-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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